

The Effect of Temperature on Expression of the Dark Phenotype in Female *Papilio glaucus* (Papilionidae)

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Abstract. Experimental broods of *Papilio glaucus* produced unusual dark morph females when reared at high temperatures. Exposure to temperatures of 25–28 C during the larval and pupal stages produced adult females which were phenotypically intermediate between the normal yellow and dark morphs of the butterfly, *i.e.*, with a dusting of yellow scales in the dark background. Naturally-occurring females with this intermediate coloration have been recorded from throughout the eastern United States, but are generally infrequent. The dark morph of *Papilio glaucus* appears to be canalized (buffered) against environmental modification under natural conditions. It is proposed that canalization of the dark morph is adaptive because it protects the mimetic resemblance of dark females to the unpalatable *Battus philenor*, and that canalization is strongest in populations of *P. glaucus* from areas where *B. philenor* is an abundant model.

Introduction

Phenotypic plasticity and polyphenism (Shapiro, 1976) in butterflies are presumably adaptive responses to heterogeneous or seasonal environments. However, developmental canalization (inflexibility of the normal phenotype over a range of environmental conditions due to the action of the epigenetic system; Waddington, 1957) is also adaptive (Shapiro, 1981; Hoffman, 1982). Phenotypic stability may be particularly advantageous in mimetic species, because environmental modification of the wing pattern would decrease the mimetic resemblance. This paper summarizes an investigation into phenotypic plasticity and canalization in the mimetic eastern tiger swallowtail, *Papilio glaucus* L.

Two subspecies of the tiger swallowtail, *P. g. glaucus* L. and *P. g. australis* Maynard, exhibit a female sex-limited wing color dimorphism (The dimorphism does not occur in *P. g. canadensis* Rothschild & Jordan). One female form resembles the male in having the typical pattern of a black-banded yellow background; the other female form is heavily melanized, with the banding pattern virtually obscured by dark scales. The dark female morph is thought to mimic the unpalatable *Battus philenor* (L.) (*e.g.*, Brower, 1958), and while both dark and yellow female morphs occur throughout the eastern U. S., the dark form is more frequent where *Battus philenor* is abundant (Brower and Brower, 1962).

Clarke and Sheppard (1957, 1959, 1962) and Clarke *et. al.* (1976) have provided compelling evidence that melanism in *Papilio glaucus* is controlled by a female-limited gene, presumably associated with the Y (W) chromosome. This is supported by the absence of the melanic form in males, and the fact that in virtually all cases, females produce daughters of the same color morph as themselves. Rare exceptions do occur in which both yellow and dark female progeny arise from a single mother (*e.g.*, Edwards, 1884; Weed, 1917; Clarke and Sheppard, 1959). Classically, these mixed broods have been regarded as the result of abnormalities in chromosome architecture or meiotic processes, but a novel explanation for certain cases was suggested by Scriber and Ritland (*in press*). These authors described a genetic component in the monomorphic subspecies *P. glaucus canadensis* that completely suppresses phenotypic expression of the dark morph in hybrid offspring from laboratory crosses between male *P. g. canadensis* and dark morph female *P. g. glaucus*. Scriber and Ritland argued that in some cases, anomalous dark morph inheritance patterns may be the result of natural hybridization between *P. g. glaucus* and *P. g. canadensis*. The rare occurrence of analagous mutant alleles in *P. g. glaucus* and *P. g. australis* may explain other cases of unusual inheritance.

Occasionally, *Papilio glaucus* females exhibit wing patterns intermediate between the normal yellow and dark morphs (*i.e.*, with a dusting of yellow scales in the dark background). The occurrence of yellow-dark intermediate individuals is an entirely separate phenomenon from the mixed broods described above. The intermediate female phenotypes are poor mimics of *Battus philenor*; Clarke and Sheppard (1959) postulated the presence of an efficient genetic "switch mechanism" in *P. glaucus* (presumably a single gene controlling melanization) which prevents the occurrence of these nonmimetic intermediates. Intermediate females of *P. glaucus* with significant yellow suffusion of the dark background are uncommon, but have been recorded from many areas in the eastern United States: New York (Edwards, 1884; Shapiro and Shapiro, 1973); New Jersey (Clarke and Clarke, 1983); Ohio (M. H. Evans, *pers. comm.*); West Virginia (Edwards, 1884); Virginia (Clark and Clark, 1951); Maryland (Clark and Clark, 1932); Pennsylvania (Shapiro, 1966; Ehle, 1981); Wisconsin (*pers. obs.*); Mississippi (B. Mather, *pers. comm.*); Kentucky (*pers. obs.*); Georgia (Harris, 1972); and Florida (*pers. obs.*). Dark morph females with at least a slight suffusion of yellow scales probably occur in low frequency throughout the eastern United States.

The general rarity of intermediate females in wild populations suggests that the dark mimetic phenotype of *P. glaucus* is strongly canalized (buffered) under normal environmental conditions. Intermediate females may arise because of either *genetic shock* (*e.g.*, mutant alleles or incomplete penetrance/expressivity of normal alleles controlling melanization) or *environmental shock* (disruption of the

canalized developmental pathway by unusual environmental conditions). The present study investigates phenotypic plasticity in dark morph *Papilio glaucus* females as a function of one environmental variable, temperature. Phenotypic plasticity and canalization of the dark morph are discussed in relation to mimicry in this butterfly. I hypothesize that canalization of the dark morph is adaptive because it stabilizes the mimetic resemblance to *Battus philenor*, and that phenotypic stability may be more strongly selected for in areas where *B. philenor* is an abundant model.

Methods

Experiments conducted in 1981, 1983 and 1984 investigated the effect of rearing temperature on wing coloration in samples of *Papilio glaucus* from eight geographic areas: Dane County, WI; Dauphin County, PA; Adams County, OH; Mercer County, WV; Bell County, KY; Jefferson County, AL; Oconee County, GA; and Alachua County, FL. Laboratory cultures were established and ova for the study were obtained from dark morph females which had been mated to male siblings by the hand-pairing method of Clarke and Sheppard (1956). Females oviposited on foodplant leaves in plastic shoeboxes warmed by incandescent lights.

Newly-eclosed larvae were transferred to environmental chambers and reared at one of three constant temperatures: 22, 25, or 28 C. Temperature readings taken at different locations within each chamber indicated fluctuations of less than 0.5 C. All treatments were maintained at a photoperiod of 16L:8D to inhibit diapause and to remove photoperiodic variability as a relevant factor. The larvae were fed leaves on excised twigs of Black Cherry, *Prunus serotina* Ehrh. Foodplant turgidity was maintained by placing the twigs in Aquapics. Pupae were kept in individual screen cages at the larval rearing temperature.

All female progeny from this experiment were expected to exhibit the normal dark morph phenotype. To describe deviation from the normal dark pattern, the dorsal background color of each reared female was scored relative to a group of five reference specimens. These reference specimens represent five points on a continuum ranging from a normal dark morph female (assigned a rating of '0') to an intermediate yellow-dark phenotype (rating = 4) which has a heavy suffusion of yellow dusting in the dark background, giving the butterfly a 'sooty' appearance (Figure 1). Reared females were compared to this reference group and assigned an appropriate score. The rating scale ranged by half steps from 0 to 4.

The modification of the dark morph pattern at different rearing temperatures was investigated statistically via the Kruskal-Wallis one-way ANOVA for ordinal data (Siegel, 1956). This procedure com-



Figure 1. Reference specimens of *Papilio glaucus* showing grading scale used to quantify dorsal wing color of experimental specimens.

pared median color rating among the three rearing temperatures within each geographic sample.

Results

A total of 281 dark morph females from the eight geographic samples were scored for dorsal wing background color. Table 1 presents the median color ratings and range of individual scores for each geographic sample at three rearing temperatures and the associated statistics. These data indicate that higher color ratings (greater suffusion of yellow scales in the dark background) occurred under high rearing temperature regimes; *i.e.*, there was significant modification of dark morph expression at 28 C relative to the two lower temperatures. In addition, the eight geographic samples differ significantly from one another in the degree of phenotypic modification at 28 C (Kruskal-Wallis ANOVA, $H = 9.4$, $p < .01$).

Intrasample variability is relatively high at 28 C: most samples reared at this temperature contained individuals ranging over at least two full steps on the color scale (Table 1). Such individual variation in susceptibility to environmental modification (or canalization of the normal dark color pattern) may represent individual differences in the suite of modifier genes which protects the normal phenotype (Waddington, 1961). Wing pattern elements other than the melanic background (*e.g.*,

Table 1. Median color scores and range of individual values for eight samples of dark female *Papilio glaucus* reared at three constant temperatures. Kruskal-Wallis test statistic (H) and significance level for differences in color rating among the three temperatures are indicated for each sample.

Sample	22 C median (range)	25 C median (range)	28 C median (range)	H	P
WI	0.0 (0.0-0.0)	0.0 (0.0-0.5)	3.0 (1.0-4.0)	16.5	.001
OH	0.0 (0.0-0.0)	0.0 (0.0-2.0)	0.5 (0.0-3.5)	8.9	.05
PA	0.0 (0.0-0.0)	0.0 (0.0-0.0)	1.8 (0.0-2.5)	21.4	.001
AL	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.8 (0.0-1.0)	11.5	.01
WV	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.3 (0.0-2.5)	4.4	.20 (N.S.)
KY	0.0 (0.0-0.0)	0.5 (0.0-2.0)	2.0 (1.0-2.5)	8.8	.05
GA	0.0 (0.0-0.0)	0.3 (0.0-0.5)	0.5 (0.0-3.5)	12.5	.01
FL	0.0 (0.0-0.0)	0.0 (0.0-1.0)	0.0 (0.0-3.0)	12.1	.01

the "tiger" stripes and wing margin borders) were virtually unaffected by temperature. The yellow forewing discal spot present in some females (see Figure 1) becomes more pronounced at higher rearing temperatures (Ritland, 1983), but varies independently of melanic background color in individual butterflies.

Discussion

Constant rearing temperatures of 25 and 28 C destabilized the dark morph phenotype of *Papilio g. glaucus* and *P. g. australis*. A previous experiment (Ritland, 1983) suggested that pattern development is susceptible to temperature modification only during the pupal stage; this is consistent with the suggestion (Clarke and Clarke, 1983) that the melanic background pattern develops just before adult eclosion. The physiological basis of aberrant intermediate pattern development is not known, but many processes involved in wing pattern development (including pigment synthesis, wing scale maturation, and hormonal control systems) are subject to modification by temperature (Goldschmidt, 1938; Hintze-Podufal, 1977; Nijhout, 1980). The temperature sensitivity of tyrosinase-mediated melanization processes in particular is well known (Waddington, 1961; Fuzeau-Braesch, 1972; Majerus, 1981), and high rearing temperatures may also disrupt the pteridine pigment system involved in *P. glaucus* pattern development (Oldroyd, 1971).

Aberrant intermediate phenotypes were expressed only in individuals reared at 25 C and above, suggesting the existence of a temperature threshold above which canalization of the normal dark phenotype breaks down. Developmental pathways are protected by such genetically-determined thresholds (Waddington, 1961), thereby canalizing the normal phenotype over a wide range of natural conditions.

This experiment did not investigate photoperiodic effects on pattern modification in *Papilio glaucus*, but photoperiod is potentially relevant

in the field. Long and short photoperiods induce different seasonal forms and aberrations in many butterfly species (e.g., Ae, 1957; Pease, 1962; Fukuda and Endo, 1966; Shapiro, 1976; but cf. McLeod, 1968 and Lewis, 1985 *re* species which are insensitive to photoperiodic manipulation).

The genetic capability to produce the intermediate phenotype represents a component of the *P. glaucus* genome which is not normally expressed, probably due to a combination of the genetic switch mechanism proposed by Clarke and Sheppard (1959) and developmental canalization. While the experimental conditions of this study (24 hr thermoperiod + 16:8 photoperiod) do not represent natural conditions, the range of rearing temperatures certainly lies within natural limits. This experiment is therefore qualitatively different from "shock" studies, in which newly-formed pupae are exposed to extreme heat or cold. Such shock treatments can produce striking pattern modifications, but also kill or cripple the majority of individuals, suggesting that critical developmental pathways are disrupted. Changes in wing pattern induced by such radical conditions may be of questionable ecological relevance. In sharp contrast to shock studies, the relatively mild conditions of the present investigation produced aberrant wing patterns but did not significantly reduce survival or adult viability (no significant difference in viability among the three temperature regimes; chi-square $p < .01$). It is significant that such moderate experimental conditions could produce such extreme phenotypic modification, given the fact that intermediates are so uncommon in the wild. This intriguing situation is similar to that described by McLeod (1968), who found that the African nymphalid *Precis octavia*, which exhibits discrete seasonal forms in nature, produced a wide variety of intermediate forms in his laboratory temperature studies.

Environmental modification of wing pattern may disrupt mimicry in dark morph *Papilio glaucus* females; the intermediate phenotypes produced at 25 and 28 C appear to be very poor mimics of *Battus philenor*. The eight geographic samples in this study differed significantly in expression of the intermediate phenotype at 28 C (Table 1). Both the proportion of aberrant individuals and the degree of phenotypic alteration varied between samples. Samples from the periphery of the dark morph range, where *Battus philenor* is uncommon (e.g., Wisconsin and Pennsylvania) were relatively susceptible to temperature modification (as indicated by the high median color ratings at 28 C). In contrast, samples from areas where *B. philenor* is abundant (West Virginia, Georgia, Alabama, north Florida) seemed to be more strongly canalized (buffered) against environmental modification. The West Virginia sample, in fact, showed no evidence of phenotypic modification by temperature.

These results are consistent with the hypothesis that canalization of the dark morph is adaptive because it stabilizes the mimetic color pattern, and that the dark phenotype is most strongly canalized in

areas where it confers the greatest mimetic advantage, *i.e.*, where *Battus philenor* is abundant as a model. In regions where *B. philenor* is rare and is therefore not an effective model, the selective advantage of the dark morph relative to the yellow morph is decreased; selection for genetic modifiers which canalize the dark morph developmental pathway should also be reduced. It is significant that many of the records for wild intermediates occur near the periphery of the dark morph range, where *B. philenor* is rare.

The occasional occurrence of wild intermediates of *P. glaucus* may be due to either environmental influences (environmental shock) or direct genetic control (genetic shock). Microhabitat selection by pupating larvae (*e.g.*, West and Hazel, 1979) may occasionally result in exposure to high temperatures which disrupt the normal dark morph developmental pathway and cause expression of the intermediate phenotype. Alternatively, mutant alleles may alter the canalization threshold of the normal dark morph (*i.e.*, change the developmental pathway), such that the intermediate phenotype is expressed under normal environmental conditions. Such alleles might be related to the gene(s) in *P. glaucus canadensis* that inhibit expression of the normal dark morph (Scriber and Ritland, *in press*). Similar inhibitory genes have been described in *Papilio rutulus*; hybrid crosses between male *P. rutulus* and dark morph female *P. glaucus* produce intermediate daughters (Clarke and Willig, 1977) that resemble the environmentally-produced intermediates (phenocopies) described in this study.

The interaction of genetic and environmental factors affecting pattern development in *Papilio glaucus* may significantly alter the resemblance to *Battus philenor*. The data presented in this paper support the hypothesis that canalization of the dark female morph stabilizes the mimetic color pattern under normal environmental conditions, and that geographic variation in the degree of phenotypic canalization is correlated with the abundance of *Battus philenor*.

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